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DR THOMAS A MORRISON (Orcid ID : 0000-0001-7262-1402)
DR RICARDO M HOLDO (Orcid ID : 0000-0002-2484-0587)

DR T. MICHAEL ANDERSON (Orcid ID : 0000-0003-0109-2528)

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Grass competition overwhelms effects of herbivores and precipitation on early tree establishment in Serengeti

Thomas A. Morrison[1,*], Ricardo M. Holdo[2], Deusdedidth M. Rugemalila[3], Mawazo Nzunda[4] and T. Michael Anderson[3]

[1]Institute of Biodiversity, Animal Health and Comparative Medicine, University of Glasgow, Glasgow, Scotland, G12 7QQ, U.K.

[2]Odum School of Ecology, University of Georgia, Athens, GA 30602-2202, USA

[3]Department of Biology, Wake Forest University, Winston Salem, NC 27106, USA

[4]Serengeti Wildlife Research Centre, Box 661, Arusha, Tanzania

[*]Correspondence author. E-mail: thomas.morrison@glasgow.ac.uk

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Summary

1. Savanna ecosystems span a diverse range of climates, edaphic conditions and disturbance regimes, the complexity of which has stimulated long-standing interest in the mechanisms that maintain tree-grass coexistence. One hypothesis suggests that tree establishment is strongly limited by one or several demographic bottlenecks at early stages of the tree life cycle. A major impediment to testing this hypothesis is the lack of data on the relative strengths of different bottlenecks across key environmental gradients.
2. To identify demographic bottlenecks that limit early tree establishment (0-18 months), we conducted a series of transplant experiments with two savanna trees species (*Acacia robusta* and *A. tortilis*) across a natural rainfall and soil fertility gradient in the Serengeti ecosystem, Tanzania. We tested the interactive effects of precipitation, herbivory, seed scarification, grass competition, water limitation and tree species identity on two key life stages: germination and early seedling survival (0-2 months) and juvenile seedling survival (2-18 months).
3. Germination and early seedling survival increased as a function of rainfall, in the absence of herbivores and when seeds were scarified. Juvenile seedling survival, in contrast, decreased with rainfall but increased in the absence of herbivores. Grass removal had the single strongest (positive) effect on juvenile seedling survival of any treatment. Soil moisture monitoring and grass-addition treatments revealed that grasses negatively affected seedlings in ways that were not necessarily linked to soil moisture.
4. A demographic model combining all effects across early life stages showed that the strength of grass competition on juvenile seedling survival was the key factor limiting early tree establishment. While rainfall had an unexpected opposing effect on the two life stages, the net effect of mean annual precipitation on early tree establishment was positive.
5. *Synthesis*: Successful tree establishment in Serengeti is maximized by a seemingly unlikely sequence of events: (1) scarification of seeds by browsers, (2) heavy rainfall to promote germination, (3) intensive grazing (but absence of browsers) and (4) dry conditions during juvenile seedling growth (>2 months) to reduce competition with grasses. By considering a wide suite of conditions and their interactions, our experimental results are relevant to ongoing debates about savanna vegetation dynamics and structural shifts in tree:grass ratios.

Key-words: *Acacia robusta*, *Acacia tortilis*, competition, environmental gradients, facilitation, herbaceous vegetation, plant-animal interactions, plant-available moisture, recruitment limitation, water

Introduction

A defining feature of the savanna biome is the discontinuous distribution of tree cover, with neighbouring trees often separated by open, grass-dominated gaps (Scholes & Archer 1997; Ratnam *et al.* 2011). Whereas grass cover is relatively uniform, tree cover is heterogeneous (Scholes & Walker 1993). The fact that tree density at many savanna sites tends to remain below its maximum potential (Sankaran *et al.* 2005) suggests that the mortality rate of established trees is high and/or that the recruitment rate of new trees is low. While considerable effort has been devoted to understanding mortality of adult savanna trees in the overstory (Pellew 1983; Asner & Levick 2012; Morrison, Holdo & Anderson 2016), far less is known about the drivers of early tree establishment. This is an important gap, given that tree establishment is a key demographic bottleneck in many forest (Clark, Macklin & Wood 1998; Clark *et al.* 1999) and savanna ecosystems (Midgley & Bond 2001). Furthermore, inferring the contribution of establishment limitation to savanna tree/grass ratios has important implications for our ability to predict biome shifts (*e.g.*, desertification or bush encroachment), as local and global environmental conditions change in ways that might favour or inhibit tree establishment (Higgins, Bond & Trollope 2000; Wigley, Bond & Hoffman 2010; Kulmatiski & Beard 2013).

Identifying the factors that shape tree establishment in savannas is made difficult by the wide range of edaphic, biotic and climatic conditions over which savannas occur. For instance, savannas persist on both sandy, nutrient-poor, well-drained soils and on clay- and nutrient-rich soils with high water retention (Scholes & Walker 1993; Sankaran, Ratnam & Hanan 2004; Lehmann *et al.* 2014). Moreover, savannas occur in areas both with and without vertebrate browsers and grazers (Goheen *et al.* 2010), and across sites where vegetation burns at various intervals, from annually to multi-decadally (Higgins *et al.* 2007). Despite this diverse environmental context, rainfall is the only consistent predictor of maximum potential tree cover in savannas at global scales (Sankaran *et al.* 2005; Lehmann *et al.* 2014), so any effort aimed at developing a framework for explaining tree demographic rates in savannas should do so in the context of variation in rainfall.

A dominant explanation for persistence of the savanna state suggests that environmental filters such as drought, herbivory and fire limit tree establishment at one or more early demographic stages (seed production, dispersal, germination and seedling survival). The removal of these filters should allow trees to outcompete and dominate grasses, potentially giving way to thickets or forests (Higgins, Bond & Trollope 2000; Sankaran, Ratnam & Hanan 2004; Sankaran *et al.* 2005). For instance, seed predation by small mammals, long ignored as a factor in the demography of savanna trees (Midgley & Bond 2001; Shaw, Keesing & Ostfeld 2002), appears to limit seed germination in a number of ecosystems (Goheen *et al.* 2004; Walters *et al.* 2005; Vaz Ferreira, Bruna & Vasconcelos 2010). At the seed germination stage, a complex suite of factors – chief among them soil moisture availability – controls rates of germination in savannas (Wilson & Witkowski 1998; Barnes 2001; Loth *et al.* 2005; Kraaij & Ward 2006). At the post-germination stage, targeted experiments have found that soil moisture availability, grass biomass, herbivory and fire affect seedling establishment rates across a range of savanna types (Hoffmann 1996; Inouye,

Allison & Johnson 2004; Tyler, Kuhn & Davis 2006; Dickie *et al.* 2007; Goheen *et al.* 2010). The suite of factors driving tree establishment also interact with one another, creating the potential for feedbacks and non-linear responses, making it problematic to examine each factor in isolation (Higgins, Bond & Trollope 2000; Kraaij & Ward 2006; van der Waal *et al.* 2009; Goheen *et al.* 2010; Grellier *et al.* 2012). So far, no study has comprehensively quantified the relative strength of these various factors for seed germination and seedling survival across a rainfall gradient.

To address this gap, we conducted a landscape-level experiment across the ~ 20,000 km^2 Serengeti savanna ecosystem, with the goal of quantifying large-scale variation in the relative importance of key demographic bottlenecks for early tree establishment (0-18 months). The Serengeti is a savanna landscape encompassing a large counter-gradient in soil moisture and soil fertility and supports one of the largest remaining intact community of large-bodied herbivores in the world (Sinclair *et al.* 2007). Previous work in this ecosystem shows that elephant herbivory and fire are dominant factors affecting juvenile and adult trees, and that adult tree mortality rates vary across tree species (Pellew 1983; Dublin, Sinclair & McGlade 1990; Morrison, Holdo & Anderson 2016). These studies have been largely or entirely observational, and they have primarily focused on the impacts of disturbance and herbivory on established adult trees. For the present study, we conducted a large transplant experiment distributed across the Serengeti rainfall/soil fertility gradient. The experiment focused on seed germination and early seedling survival (0-2 months, hereafter 'seedling recruitment') and juvenile seedling survival and growth (2-18 months, hereafter 'seedling survival'), and involved two dominant tree species, *Acacia robusta* (= *Vachellia robusta*) and *Acacia tortilis* (= *V. tortilis*) (Rugemalila *et al.* 2017)), whose ranges overlap partially in Serengeti. In addition to exploiting the rainfall/soil fertility gradient, the experiment was designed to manipulate seed scarification and predation at the seed germination stage, and browsing, grass competition, water availability at the seedling stage.

We tested explicit hypotheses for each life stage. Given the importance of seed predation by small mammals in other African savanna systems (Goheen *et al.* 2010), we hypothesized that germination success and early seedling survival would increase strongly when seed predators were excluded. We also hypothesized that germination success and early seedling survival would increase as a function of rainfall, and that the rainfall effect on this stage would vary by tree species. We expected that the tree species found in wetter areas, *A. robusta*, would be limited more by germination success at dry sites than *A. tortilis,* but that both tree species would have equivalent germination success in wet sites.

At the juvenile seedling survival stage, we hypothesized that water availability would limit seedling growth and survival rates at the dry end of the rainfall gradient. Previous work shows that wetter areas have higher densities of understory trees (Holdo, Anderson & Morrison 2014). We therefore predicted that growth rates and survival in high rainfall areas would be more strongly limited by top-down (*i.e.*, herbivory) effects than by water limitation. We also tested the alternative hypothesis that grasses limit seedling survival across the entire rainfall gradient. We expected that grass removal would promote juvenile seedling survival by reducing grass competition (Grellier *et al.* 2012). We based this hypothesis on recent work

suggesting that grasses exert strong competitive effects on savanna tree growth rates, even under elevated precipitation (February *et al.* 2013).

Ultimately, our goal was to combine all experimental results into a single demographic framework to understand the conditions that maximize savanna tree establishment. By considering the interactive effects of a variety of abiotic factors, this model revealed which of the two early life stages act most strongly as a demographic bottleneck during tree establishment.

Materials and methods

STUDY AREA

Our study sites spanned Serengeti National Park and Ngorongoro Conservation Area in North-western Tanzania, which together form part of the larger Serengeti Ecosystem (*ca.* 25,000 km^2), hereafter 'Serengeti' (Sinclair *et al.* 2007). Serengeti consists of grasslands in the southeast, where soils underlain by a calcareous 'hard-pan' prevent tree root systems from developing, and the savanna woodland, which comprises roughly two-thirds of the park in the central, western and northern sections of the ecosystem (Reed *et al.* 2009). The ecosystem spans general gradients in rainfall (~500 to >1000 mm yr^{-1}) and soil fertility from the NW (high rainfall, low soil fertility) to the SE (low rainfall, high soil fertility), the combination of which helps drive the famed wildebeest (*Connochaetes taurinus*) migration (Holdo, Holt & Fryxell 2009). Serengeti supports a diverse community of other large-bodied mammalian herbivores, including ruminant livestock that occur in Ngorongoro Conservation Area. Woody cover in Serengeti has changed substantially over the past century (Sinclair *et al.* 2008). During the late 1970's and early 1980's, declining elephant densities and a reduction in dry season fire frequency coincided with rapid ecosystem-wide increases in tree recruitment (Sinclair *et al.* 2007). Two tree species – *A. tortilis* and *A. robusta* – presently dominate the Serengeti woodland overstory, account for 60.1% of all individual trees in long-term woodland plots (Anderson *et al.* 2015; Rugemalila *et al.* 2017). Although the ranges of these species overlap in central Serengeti, *A. tortilis* is more strongly associated with drier sites, while *A. robusta* dominates in higher rainfall sites.

EXPERIMENTAL DESIGN

Our main experiments examined the role of water limitation and biotic factors on two early demographic stages: (1) seed germination and early seedling survival (0-2 months; hereafter 'seed germination') and (2) juvenile seedling mortality and growth (2-18 months, 'hereafter 'seedling survival').

We used two variables to characterize rainfall. We obtained daily rainfall data from the African Rainfall Climatology, version 2 (ARC2) daily rainfall product from NOAA (Novella & Thiaw 2013). The ARC2 product integrates rain gauge data with satellite-based microwave data, and is available at a 0.1° spatial resolution and a daily temporal resolution across the

African continent (Herman *et al.* 1997; Novella & Thiaw 2013). We generated layers spanning the duration of germination and early seedling survival (April-June 2015) and juvenile seedling survival experiments (February 2014 – May 2015) and calculated separate mean daily rainfall amounts (variables *RAIN_GERM* and *RAIN_SEEDL*, respectively) across the Serengeti. For each experimental plot, we extracted rainfall values from the resulting rasters. We also estimated mean annual precipitation (variable *MAP* in the analysis) for each plot based on interpolated monthly rainfall records collected at 58 rain gauge locations across Serengeti National Park from 1960-2006 by the Serengeti Ecology Department (Coughenour 2006). *MAP* values were estimated using inverse distance weighting, after correcting for effects of elevation.

We established 38 20m x 50m (0.1-ha) plots across the Serengeti rainfall gradient (Holdo, Anderson & Morrison 2014; Anderson *et al.* 2015), grouped into 10 sites (variable *SITE*) with four plots per site, except at our driest site (Ngorongoro Conservation Area) which had only two plots. At each site, half of the plots were randomly assigned to the seed germination-early seedling survival experiment and half of the plots were assigned to the juvenile seedling experiment. Plots ranged in *MAP* values between 592 to 952 mm yr^{-1}.

Within each plot, we randomly established either eight or ten 1-m^2 subplots (*SUBPLOT*; Figs. 1b & S1). The number of subplots per plot depended on whether the plot was assigned to seeds (n=8 subplots) or seedlings (n=10 subplots). Subplots were located in open areas of plots away from the edge of tree canopies to avoid negative effects of adult *Acacias* on tree establishment (Loth *et al.* 2005). Furthermore, one of our main goals was to determine the factors that limit tree establishment in areas that lacked trees. Experimental treatments were applied to these subplots in a semi-crossed factorial design (Fig. 1b; *see* below).

EXPERIMENT 1: SEED TRANSPLANTS (0-2 MONTHS)

We collected *Acacia* seeds for all experiments by opportunistically gathering mature seed pods from 40 adult individuals of *A. robusta* and 21 adult individuals of *A. tortilis* across the Serengeti woodlands in 2012 and 2013, with the goal of maximizing spatial coverage of fruiting trees. We pooled seeds within four regions that corresponded approximately to areas North, East, South and West of the research centre in central Serengeti. We discarded seeds that were abnormal or infested by bruchid beetles. In a concurrent study (Rugemalila *et al.* 2017) we found no differences in seed infestation and viability rates across the rainfall/fertility gradient in Serengeti (using the same pool of seeds as this study). For both seed and transplant experiments, we matched experimental seeds and seedlings to the general region of seed collection to minimize potential confounding effects of transplanting individuals outside their adaptive ranges (see Rugemalila *et al.* 2017).

Half of the subplots within each plot were open to seed predation/herbivory. In the other subplots, we excluded seed predators by constructing 1-m^2 pyramid-shaped herbivore exclosures (variable *CAGE*; see Fig. S1 in Supporting Information). Exclosures were first covered in 'chicken wire' to exclude large herbivores. Mesh wire (1-cm^2) was then attached

to the lower sections of the exclosures (≤15 cm) to exclude small herbivores and seed predators. Identical exclosures were constructed in half of all subplots in the seedling transplant experiment. Notably, the exclosures were designed to measure the net effects of herbivory on tree establishment, which include direct effects of seed predation and browsing, and indirect effects of changes in grass biomass (i.e. increased grass biomass in exclosures) and changes in the micro-environment. These net effects are unlikely to be separated in natural systems, so we did not attempt to manipulate grass biomass/microclimate within exclosures.

Seed scarification is a potentially limiting factor in *Acacia* trees, as seeds and seed pods are often consumed by mammalian and avian species, and the abrasion caused by gut passage appears to facilitate germination rates through more rapid emergence of the cotyledon (Or & Ward 2003). Thus, half of all seeds in the seed transplant experiment were scarified immediately prior to planting (variable *SCAR*) using a razorblade to remove a small section of the seed coat (Rugemalila *et al.* 2017).

We planted 16 seeds in each subplot of the seed transplant experiment, yielding a total of $N = 2432$ seeds across the experiment (16 seeds x 8 subplots x 19 plots). Eight of the sixteen seeds within subplots were scarified while eight were un-scarified (Fig. 1b). Further, each subplot contained seeds from only one tree species (either *A. tortilis* or *A. robusta*) which was randomly assigned within plots (Fig. 1b). We transplanted seeds to the field in April 2015 and we subsequently visited subplots twice post-planting (in weeks 4 and 6 from the date of planting) to check for new seed germination. This design allowed us to evaluate the effects of four treatments (combinations of ±*CAGE* and ±*SCAR*) on the probability of seed germination and early seedling survival in each of the two tree species (Fig. 1b).

We had initially planned to include an experimental fire treatment in both the seed and seedling experiments, but extensive early grazing and subsequent loss of fuel load in 2014 prevented experimental burning. Thus, we did not consider fire in the analysis and focused on the effects of rainfall, grass competition, herbivory and their interactions.

EXPERIMENT 2: JUVENILE SEEDLING TRANSPLANTS (2-18 MONTHS)

In January 2014, we began growing seedlings for transplantation by germinating seeds in a protected nursery located in central Serengeti under common water and light conditions. Six to eight weeks after germination (February-March 2014), we transplanted seedlings to our 10 sites across the Serengeti. Within each 1-m^2 subplot, we planted two *A. tortilis* and two *A. robusta* seedlings (Fig. 1b). We planted seedlings during the long rainy season, shortly after rainfall events had occurred, and saturated the soil with supplementary water at the time of planting. We replaced seedlings that died within 2.5 months of the beginning of the experiment. Thus, some locations had up to three cohorts (variable *COHORT* in the analysis) of seedlings during the early transplant stage. In total, we planted 1182 seedlings ($N_{cohort\ 1}$ =

760, $N_{cohort\ 2}$ = 382 and $N_{cohort\ 3}$ = 40). We revisited plots every 4-6 weeks after planting to assess seedling survival and to measure soil moisture availability. During each visit, we measured the basal diameter of living seedlings using hand-held callipers to determine growth rates. No measurements of basal area were recorded during the first two plot visits because stems were too fragile for calliper measurements. This may have introduced some bias into our early growth data (*i.e.*, only those seedlings still alive after 2 visits (roughly two months post-transplant) were available for basal measurements, n = 346 plants). However, we collected basal measurements for 14 months, so the majority of basal growth occurred during the measurement period.

As in the seed transplant experiment, our seedling transplant experiment tested for the effects of herbivory (*CAGE*) and rainfall (*RAIN_SEEDL*) on juvenile seedling survival, but also included grass competition (*GRASS*) and water addition (*WATER*) treatments. To test for the effect of grass competition on juvenile seedlings, we completely removed grasses from two subplots per plot (-*GRASS*) by digging below the grass root mass (~5 cm below the soil surface) and discarding all grass material including stems, roots and invading stolons. During subsequent visits to the plot we removed any new grass growth. To study water limitation, we added ~10 L of water to each water addition subplot (+*WATER*; Fig. 1b) during visits in the dry season, resulting in 3-4 watering events from June-October equivalent to 50 mm of additional annual rainfall subplot^{-1}. We watered two caged and two uncaged subplots per plot. The remaining plots received no supplemental water (-*WATER*). We collected and stored rainwater for this treatment in polyurethane tanks located throughout the park.

To keep the seedling transplant experiment tractable, water addition was not crossed with the grass removal (i.e. there was no -*GRASS+WATER* treatment). In total, each plot in the seedling transplant experiment contained six different treatment combinations: (i) -*GRASS+CAGE-WATER,* (ii) -*GRASS-CAGE-WATER,* (iii) +*GRASS+CAGE-WATER*, (iv) +*GRASS-CAGE-WATER* (=control), (v) +*GRASS+CAGE+WATER,* (vi) +*GRASS-CAGE+WATER*. Each treatment combination had two replicate subplots per plot, except for the grass removal treatments (**-***GRASS*), which had only a single replicate per plot (see Fig. 1b).

One of the most plausible mechanisms by which grass and herbivory impact seedling survival is through its effect on soil moisture. Because quantifying soil moisture continuously over the course of the seedling transplant experiment in every subplot was logistically unmanageable, we measured volumetric water content (*VWC*) during each visit to subplots (prior to the water addition treatment) using a handheld time-domain reflectometry soil moisture probe at a depth of 10 cm (CS659, Campbell Scientific, Logan, UT) and conducted *post-hoc* analyses of *VWC* across different treatments and versus rainfall. *VWC* values were highly dynamic over time so we did not include them as covariates in our germination and survival models, but rather analysed their deviation from mean values separately to understand gross treatment and rainfall effects.

EXPERIMENT 3: GRASS ADDITION

We conducted a separate juvenile seedling transplant experiment to understand the specific competitive effects of grasses on seedlings. The goal of this experiment was to differentiate belowground competition (e.g., soil moisture and nutrients) and aboveground competition (e.g., light or increased herbivory by insects living in, or attracted to, the grass matrix), and their relative effects on juvenile seedling survival. Thus, we planted seedlings in three grass treatments: removal (-*GRASS*), addition (+*GRASSADD*), and a control (*i.e.*, no grass manipulation, + *GRASS*). For the grass addition treatment, we first removed grass and then placed the exhumed grass clumps back into the addition subplots, surrounding the target seedlings. This preserved above-ground grass cover while removing the below-ground competitive effects of grasses. Subsequent observations confirmed that grass clumps did not regrow roots or shoots during the experiment. If grasses affected seedling survival through belowground competition, we expected equivalent survival rates in the grass removal and grass addition subplots. If grasses affected seedling survival through aboveground competition, we expected reduced survival rates in the grass addition treatment.

We were interested in how grass competition interacted with herbivory, so crossed the three grass treatments (±*GRASS* and +*GRASSADD*) with herbivory treatments (±*CAGE*), yielding six treatments in total. Small exclosure cages were constructed using fine mesh wire (~1 cm^2) bent to form boxes that surrounded the seedlings. We established each of the six treatments at eight woodland sites within 2 km of the Serengeti Wildlife Research Centre in Central Serengeti. We planted a single individual of each focal tree species (*A. robusta* and *A. tortilis*) in each treatment plot using the same planting protocol as the large seedling transplant experiment. The experiment began immediately after a period of heavy rainfall on April 2, 2014 and continued for 16 weeks, so was considerably shorter than the main juvenile seedling experiment (Experiment 2). The area received only trace amounts of rainfall during this time and there was no water supplementation. Seedling survival, growth and soil moisture were measured weekly using the methods described above in the seedling transplant experiment.

ANALYSES

To analyse germination success and early seedling survival (Experiment 1), we used generalized linear mixed-effect models assuming a binomial error distribution and fit using maximum likelihood. We fit separate models to data from our first and second visits following seed planting (month 1 and month 2 post-transplant). We treated *CAGE*, *SCAR*, *SPECIES* and *RAIN* (and interactions between *RAIN* and *CAGE*, *SCAR* and *SPECIES*) as fixed effects, *PLOT* nested within *SITE* as random effects. To facilitate model convergence, we rescaled *RAIN* to have a mean = 0 and a standard deviation = 1.

For juvenile seedling survival data (Experiment 2), we fit a mixed-effect 'counting process' Cox-proportional hazards model (package '*coxme'* in program R) to our seedling data (R: Development 2012). This approach models the time to event (*i.e.*, mortality) as a

function of predictor variables, while allowing for the inclusion of random effects. To account for the fact that multiple cohorts were used in this experiment, we proceeded in two stages. First, we tested for treatment effects on Cohort 1 seedlings only. We included *SPECIES*, *GRASS*, *CAGE*, *WATER* and *RAIN* as main effects and *CAGE*:*GRASS*, *CAGE*:*WATER* and *SPECIES*:*GRASS* as ecologically relevant interaction effects in the model. We treated *SUBPLOT* nested within *PLOT* (in turn nested within *SITE*) as random effects. To account for the fact that additional seedling cohorts were subsequently planted, we repeated the analysis with all three cohorts, but included an additional random effect for *COHORT*. The additional cohorts can be thought of as a selective increase in sample size, leading to an unbalanced design. Our analysis accounts for potential non-independence issues by modelling random location (through *SUBPLOT*) and time effects (through variable *COHORT*). We derived stem basal area from stem diameter measurements and converted it to a rate by calculating the difference in basal area over time. We analysed basal area growth rates using a linear mixed model with fixed and mixed effects, as in the seedling survival model.

In the grass addition experiment (Experiment 3), we again fit survival data to a Cox proportional survival model with mixed effects, and tested for the effects of grass removal, grass addition and grass control crossed with cage and no cage. We specified *PLANT-ID* nested within *SITE* as random effects. As with the larger seedling survival experiment, we fit a single model because we were mainly interested in using the experiment to understand effects of grass competition on seedling survival above- and belowground.

A final goal was to estimate the probability of a single seed germinating and surviving to establish as a seedling. We combined results from models from Experiments 1-2 to predict the probability of a single seed establishing across factors that were important in our models (*SPECIES, SCAR, CAGE, GRASS* and mean annual precipitation, *MAP*). In this analysis we relied on *MAP* instead of satellite-derived rainfall because we were interested in mean responses to precipitation that would permit comparisons to other savannas. *MAP* and satellite-derived rainfall in our study were highly correlated *(RAIN_GERM*: r^2=0.896, *RAIN_SEEDL*: r^2=0.818) so qualitative patterns remained the same regardless of the precipitation metric used. We used a bootstrapping approach with 1000 iterations to estimate 95% confidence intervals from each combination of experimental treatments. For simplicity, we ignored random effects, given that our main purpose was to achieve general predictions rather than differences based on site- or plot-specific random effects.

Results

SEED GERMINATION AND EARLY SEEDLING SURVIVAL (0-2 MONTHS)

We detected strong positive effects of seed scarification, caging and rainfall on *Acacia* germination success and early seedling survival over both survey periods (Table 1, cumulative germination as measured in the second survey). Scarification was a dominant factor, increasing the odds of germination by a factor of 4.7 (Table 1). The positive rainfall

coefficient suggested an approximate doubling in germination success and early seedling survival for every standard deviation increase in precipitation (Table 1, Fig. 2). Similarly, caging increased the odds of germination and early seedling survival by a factor of 1.6 (Table 1). We found no support for an interaction between species and rainfall in the first survey (four weeks post seed planting) and only weak support for an interaction in the second survey (eight weeks post seed planting; Table 1). We found no evidence for differences in seed germination success and early seedling survival between tree species (*A. tortilis* vs. *A. robusta*) nor were there interactions between species identity and other effects, suggesting that the effects of rainfall and caging from herbivores and seed predators were additive. To better understand the rainfall effect, we investigated the relationship between mean volumetric water content (*VWC*) measured at the subplot scale and averaged across all visits over the course of the wet season, and *RAIN_GERM*, measured at the plot level, using a mixed effects regression of log(*VWC*) vs. log(*RAIN_GERM*) with *PLOT* within *SITE* as random effect using the *lme* function in the *nlme* package (Pinheiro and Bates 2005). We found a significant positive relationship ($\beta_{\log(RAIN\_GERM)} = 0.455 \pm 0.126$, $P < 0.05$, Fig. S2), suggesting that the positive *RAIN_GERM* effect may be acting via effects on soil moisture availability rather than through other correlated (but unmeasured) processes.

JUVENILE SEEDLING SURVIVAL (2-18 MONTHS)

The analysis of the first seedling cohort, as well as the analysis of all cohorts together, identified the same factors as significant, so we report the latter results here. There were clear effects of *SPECIES*, *CAGE*, *GRASS* and *RAIN_SEEDL*, and *SPECIES*:*GRASS* on juvenile seedling survival, but no effects of *WATER* or of other interaction effects (Table 2). We found that the presence of grasses relative to grass removal increased the relative risk of juvenile seedling mortality more than threefold (Table 2), while protection from herbivores via caging reduced the relative risk by half. *A. tortilis* had higher survivorship than *A. robusta* when grass was removed, but lower baseline survivorship when grass was present, suggesting a higher susceptibility to competition from grasses in *A. tortilis* (Fig. 3). Overall, the results showed dominant effects of grass competition exceeding the effects of herbivores on juvenile seedling survival.

In contrast to Experiment 1, we found that increased rainfall decreased juvenile seedling survival (Table 2). To investigate any potential role of soil moisture availability not captured by rainfall, we analysed the effects of the various experimental treatments on our *VWC* data. To account for the highly dynamic nature of *VWC* or time (*e.g.*, in response to rainfall events that may have occurred in some plots but not others just before measurements were conducted), we normalized our *VWC* data by subtracting subplot *VWC* values from mean *VWC* calculated at the plot level for each visit. We then tested for treatment effects on these *VWC* deviations from plot means using a linear mixed effects model with R package '*nlme*', treating *PLOT* within *SITE* as random effects. Our analysis found that caging had a positive effect on the *VWC* deviation ($\beta_{+CAGE} = 0.626 \pm 0.291$, $P < 0.05$) while grass removal had a negative effect ($\beta_{-GRASS} = -0.828 \pm 0.291$, $P < 0.05$) and water addition had no discernable

effect (note that *VWC* data were collected prior to water addition, Fig. S3). Given that grass removal was the dominant driver of juvenile seedling survivorship but had a negative effect on *VWC*, our results from a hand-held TDR sensor of soil moisture did not support the notion that the competitive effects of grasses on seedlings was mediated through soil moisture.

Basal area growth rates of juvenile seedlings were unaffected by caging, water addition and rainfall (Table 2). Basal growth rates were significantly higher in *A. tortilis* than *A. robusta*, though this difference was far greater in the grass removal subplots (Table 2), suggesting that grass competition more strongly reduces growth in *A. tortilis* than in *A. robusta*.

GRASS ADDITION EXPERIMENT

The grass addition experiment examined in further detail the mechanisms causing higher juvenile seedling survivorship when grass was absent. Specifically, we wanted to establish whether the effect was caused by competition for soil moisture *vs.* other micro-environmental effects related to the presence or absence of grass (*e.g.*, shading, attraction to the grass matrix by insect herbivores). Among 96 transplanted seedlings, grass removal and grass addition treatments both reduced mortality risk of seedlings by nearly a quarter relative to the control (no grass removal) (P = 0.01 and P = 0.01, respectively; Table S1 in Supporting Information). Caging reduced mortality risk more (*i.e.* seedlings survived better) when grass was removed than when grass was added ($\beta_{+Cage-Grass}$ = -1.726 ± 0.554, P = 0.002 *vs.* $\beta_{+Cage+GrassAdd}$ = -1.154 ± 0.554, P = 0.03; Table S1), and this interaction translated into higher overall survivorship in grass removal than in grass addition subplots (Fig 4). Despite the survival difference between grass removal and grass addition, we found no difference in soil moisture (*VWC*) between these treatments (Fig. S4), which again suggests that grasses (whether roots are intact or not) impact seedling survival in ways that are at least somewhat unrelated to soil moisture.

PREDICTED PROBABILITY OF ESTABLISHMENT

Our combined results from models of seed germination and seedling survival (including only significant covariates in each case) allowed us to jointly model the effects of demographic bottlenecks occurring at the seed germination-early seedling survival stage and the juvenile seedling stage. Grass removal and seed scarification had the strongest positive effects on the probability of *Acacia* establishment (Fig. 5). Without grass removal, seedling establishment was predicted to be extremely low (< 3%). The relatively strong positive effect of rainfall on seed germination resulted in a net positive effect of *MAP* on establishment, despite the negative relationship between rainfall and seedling survival. At low rainfall (< 600 mm yr^{-1}), the relative difference in establishment between trees with scarified versus un-scarified seeds, or grass-removal versus control plots, was relatively small, as most trees failed to become established. This was primarily due to low seed germination and low early seedling survival at dry sites. Thus, high rainfall enhanced the positive effects of grass removal and seed

scarification, relative to controls. Finally, because of higher survival in *A. tortilis* juvenile seedlings than *A. robusta*, establishment rates of *A. tortilis* were also predicted to be higher.

Discussion

Our study quantifies – for the first time – the relative importance of multiple bottlenecks for savanna tree establishment across a wide range of rainfall. Of these, competition between grasses and seedlings appears to impose the single strongest demographic filter on early tree establishment (Fig. 5) and therefore is likely also the main determinant to tree density in the understory woody herbaceous layer (Anderson *et al.* 2015). Even under the best growing conditions in our experiment (+*SCAR*, +*CAGE*, high rainfall, *A. tortilis*), the predicted establishment rate was 3.2% in the presence of grass, and 31.9% without grass (Fig. 5). This provides further compelling evidence of the importance of grass competition in structuring savannas (Riginos & Young 2007; Riginos 2009; Grellier *et al.* 2012; February *et al.* 2013).

The notion that grasses limit seedling establishment is not new, but empirical studies that quantify this effect are surprisingly scarce (Davis *et al.* 1999; Dickie *et al.* 2007; Hagenah *et al.* 2009). Experimental studies in African savannas have shown that grass competition has clear negative effects on the growth of established trees across a range of demographic stages (Riginos & Young 2007; Riginos 2009; Grellier *et al.* 2012; February *et al.* 2013). Nonetheless, while grasses reduce tree growth rates, they do not entirely arrest growth, suggesting that in the absence of fire or browsing, the biomass of established trees could eventually reach some maximum value that is independent of the grass effect (Kraaij & Ward 2006). In other words, the effects of grass on growth only delay the inevitable, whereas the large effects of grass on juvenile seedling survival potentially prevents tree colonization in grass-dominated patches over prolonged periods of time.

Several mechanisms may underlie competition between grasses and trees. In terms of water acquisition, C_4 grasses appear to have a competitive advantage over trees during wet periods, and can transpire at ~five times the rate of trees (Xu, Medvigya & Rodriguez-Iturbe 2015). In an elegant experiment in Kruger National Park, February *et al.* (2013) showed that sapling growth of savanna trees was unaffected by increasing amounts of rainfall when grasses were present, suggesting that 'excess' water inputs tend to accrue to grasses not trees. Watering experiments in Serengeti have similarly demonstrated that C4 tall grasses are major water 'spenders,' showing that these grasses can rapidly (*i.e.* within a few days) deplete soil moisture after rainfall events (Williams *et al.* 1998), presumably to the detriment of slower growing competitors, such as trees. Our results add to these findings: we found no effect of water addition on juvenile seedling survival, and survival was highest in low rainfall areas. This counter-intuitive result supports the notion that the overall effect of increasing rainfall may be to favour grass growth to the detriment of tree survival at this demographic life stage.

Our results are also consistent with the notion that Serengeti grasses compete with trees for resources other than soil moisture. For example, Cramer, Wakeling & Bond (2012) found that tree seedlings growing with grass competitors responded positively to fertilization, while

seedlings growing without grasses did not, suggesting that tree seedlings and grasses may be competing for soil nutrients (though *see* Kraaij & Ward (2010) for opposite effects). In fact the Serengeti rainfall gradient encompasses a counter-gradient in soil fertility and the driest areas are both the most fertile and have the least biomass, which enables more light penetration to the soil surface (Anderson *et al.* 2010). Thus, the negative effects of rainfall on seedlings in our study could be driven at least partly by positive (but unmeasured) effects of soil fertility and light. Despite this possibility, Cramer, Wakeling & Bond (2012) also found that i) even high rates of fertilization failed to fully compensate for the grass competitive effect, and ii) similar competitive responses occurred in N_2-fixing and non-N_2-fixing tree species. As an alternative hypothesis for the grass effect on tree seedlings, the authors proposed competition via mechanisms other than soil nutrients and soil moisture whereby dense grass roots impose spatial constraints that limit the ability of tree seedling roots to fully develop and exploit the available soil volume, (Cramer, Wakeling & Bond 2012). This interpretation is supported by recent work showing that grassland-to-savanna transitions are accompanied by a dramatic increase in the number of gaps in the grass root layer that are suitable for tree seedling establishment (Wakeling *et al.* 2015). In addition to these belowground effects, we found evidence for aboveground grass-seedling interactions. Our grass addition experiment (Experiment 3) showed that seedling survival was higher when grass was completely removed, as opposed to removed then added back to a plot (Fig. 4). This suggests that grass competition acts partly through aboveground mechanisms, such as through light competition or by providing habitat for insect herbivores.

We found an intriguing contrast in the effects of rainfall on early tree stages in our study: seed germination and early survival of germinants were strongly dependent on wet conditions (Fig. 2) while juvenile seedling survivorship was higher in dry areas (Hoffmann 1996; Kraaij & Ward 2006). We suspect the positive rainfall effect on germination and very early survival in our study was mediated through soil moisture (Fig. S2). Seed scarification strongly enhances germination rates (Fig. 5), as water more rapidly infiltrates the seed coat, allowing seeds to respond quickly when soils are wet (Or & Ward 2003). Thus, the importance of water for tree establishment becomes clear when incorporating germination dynamics: in areas with consistently low rainfall, seed germination becomes the critical demographic bottleneck, regardless of other factors such as grass presence and herbivores (Fig. 5; (Rugemalila *et al.* 2017)). Our results imply that trees benefit from wet conditions during germination and drier conditions once juvenile seedlings have become established, possibly because of the impact water has on grass competition (February *et al.* 2013). The timing of the transition between trees favouring wet versus dry conditions is unclear, but our transplanted seedling experiments (Experiment 2-3) suggests it may occur ~2-3 months post-germination.

Both *A. tortilis* and *A. robusta* germinated and survived in plots outside their current ranges within Serengeti, suggesting early life stages do not constrain the species' respective distributions. *A. tortilis* and *A. robusta* overlap spatially in many portions of the park, though *A. tortilis* is generally more common in drier areas (550-900 mm yr^{-1}) and *A. robusta* in wetter areas (650-1000 mm yr^{-1}). These broad distribution patterns correspond well with our

experimental results: the grass removal treatment had a stronger positive effect on growth and survival of *A. tortilis* than on *A. robusta* (Tables 2 & 3). Thus, *A. tortilis* appears to be more sensitive to grass competition, but in the absence of grass, *A. tortilis* can better tolerate dry conditions. Given its overall lower survival across treatments, *A. robusta* also appears to be more strongly limited by early demographic bottlenecks in Serengeti than *A. tortilis*.

The exclusion of herbivores and seed predators positively affected early tree establishment. Several direct and indirect pathways may underlie this effect (Shaw, Keesing & Ostfeld 2002), including higher germination success in exclosures due to reduced seed predation (direct) or cage effects on the soil and surface micro-environment (Goheen *et al.* 2010). Anecdotally, we found little sign of granivory, which contrasts with germination trials in other East African savannas (Goheen *et al.* 2010). We excavated a subset of sites in which seeds had been planted and found that a large majority of un-germinated seeds were still present in the soil, but that they had swollen and rotted, rendering them non-viable (*unpublished data*). This suggests that failure of seeds to germinate and grow to the end of the experiment (2 months post planting) was not due to granivory or herbivory on germinants, but rather to seed inviability. The heavy grazing that occurred in some of our plots may have suppressed local seed predator abundances and in turn reduced the impact of seed predation (Maclean *et al.* 2011).The effects of cages on seedling survival were potentially more direct: many juvenile seedlings outside of cages were trampled or disappeared completely, presumably indicating browsing. If grazing reduces grass-seedling competition in ways that indirectly enhance juvenile seedling survival, as suggested by February *et al.* (2013), these effects appear to be swamped by the direct impact of browsing on seedlings. Unfortunately, we were unable to separate direct browsing from indirect grazing effects in our experiment, but the net effect of herbivory was negative (Fig. 5).

The roughly two million large mammalian grazers in Serengeti, along with high fire frequency, strongly moderates grass biomass in Serengeti, and many sites become nearly bare during the dry season(McNaughton 1985). Despite the negative effects of browsing on seedlings, the potential reduction in grass-seedling competition and fire in these bare sites should enhance seedling establishment. This mechanism may partly underlie the apparent trophic cascade that occurred in Serengeti beginning in the 1970's, where tree cover increased during a relatively wet period when wildebeest numbers increased and the fire regime changed from hot, late season (August-October) fires to early, cool season (June-July) fires. If grazing controls tree establishment we would expect high seedling recruitment every year, yet the Serengeti trophic cascade appears to have been a pulsed recruitment event. This suggests other mechanisms, such as seed limitation (*e.g.* Rugemalila et al. 2017), may play important roles in the dynamics of understorey trees in Serengeti.

The effects of fire on savanna tree seedlings are relatively well understood (Trollope 1984; Higgins, Bond & Trollope 2000; Grellier *et al.* 2012), including in Serengeti (Pellew 1983) though fire was not part of our experiment. Fires impose high, direct mortality in first-year seedlings when fires are hot and frequent, particularly when seedlings have failed to develop sufficient bark thickness (Trollope 1980). The dominant effects of grasses on seedling survival at our site suggest that fires could benefit seedlings under certain

conditions, for example if burning occurs at low intensity and reduces the competitive effects of grasses on soil moisture (Grellier *et al.* 2012) after a period when seedling have developed fire-resistant bark. Thus, at the ecosystem scale, we suspect the combination of heavy grazing and frequent, low-intensity fires may favour tree growth through suppression of grass competition and that these processes underlie recent increasing densities of understory trees in Serengeti (Holdo, Anderson & Morrison 2014).

Conclusions

Our results build a more complete picture of the complex set of interactions that underlie tree-grass coexistence in savannas, and suggest that tree establishment is maximized when (1) seeds are scarified, (2) germinate and grow as seedlings in high rainfall sites that are free from herbivores (including seed predators), but (3) grow as juvenile seedlings in the absence of grasses in dry areas that are free of browsers. This set of ideal establishment conditions poses several potentially conflicting requirements for savannas trees. For example, the positive effect of seed scarification in *Acacia* requires the passage of seeds through intestinal tracks of large herbivores, such as elephants and giraffe (Or & Ward 2003), but these herbivores are also important consumers of seedlings, so their presence in an area likely reduces early seeding survival. Further, high rainfall is a net benefit to tree establishment – a pattern that is consistent with higher relative densities of understory trees in higher rainfall areas of Serengeti (Holdo, Anderson & Morrison 2014) – because of its positive effect on seed germination and early seedling survival (Fig. 6). However higher rainfall promotes grass production and is negatively associated with juvenile seedling survival (February *et al.* 2013), so wet periods for germination must be followed by dry periods to promote juvenile seedlings. Finally, grazers (and potentially low-intensity fires) act as important modifiers of grass biomass by reducing the growth potential of grasses and therefore reducing competition between grasses and trees (Goheen *et al.* 2010). Thus, savanna trees must pass through a series of demographic bottlenecks to establish in particular sites (Sankaran, Ratnam & Hanan 2008; Holdo, Anderson & Morrison 2014) and these bottlenecks can be opposing at different life stages. Despite the apparent low probability of passing these bottlenecks, tree and bush densities are increasing in many parts of Africa (Wigley, Bond & Hoffman 2010), including in Serengeti (Holdo, Anderson & Morrison 2014). Our study provides important context for understanding these changes, and suggests that shifts in rainfall and herbivory regimes, via associated impacts on grass competition, can alter tree demography in ways that may have consequences for the structure and dynamics of woody cover.

Authors' contributions

TAM, RMH and TMA designed the experiments. TAM, RMH, DMR, MN and TMA contributed to field work, data collection and conceptualization of results. TMA, RMH and TAM analysed data. TAM wrote first draft of the manuscript, and all authors contributed critically to subsequent drafts.

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Data accessibility

Data available from the Dryad Digital Repository https://doi.org/10.5061/dryad.tn2d16t

(Morrison *et al.* 2018).

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Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Effect of grass addition on seedling survival.

Fig. S1 Image of the $1m^2$ pyramid cages used to exclude herbivores in the seed transplant and seedling transplant experiments (Experiments 1-2).

Fig. S2 Comparison of mean soil moisture (volumetric water content) across the rainfall gradient in the seed transplant study (Experiment 1).

Fig. S3 Comparison of soil moisture (VWC) across six experimental treatments in seedling transplant study (Experiment 2).

Fig. S4. Comparison of soil moisture (VWC) across six experimental treatments in grass addition experiment (Experiment 3), showing no difference between grass removal and grass addition treatments.

TABLES

Table 1. Effects of exclosures (+*CAGE* vs. control -*CAGE*), seed scarification (+*SCAR* vs. control -*SCAR*), rainfall and species identity (*Acacia tortilis* vs. *A. robusta*) on seed germination success and early seedling survival across the Serengeti ecosystem inferred a using general linear mixed-effects model.

Factor	β	SE	exp(β) ‡	Z	P-value
$SPPS_{A.tortilis}$	0.123	0.118	1.154	1.21	0.226
+*CAGE*	0.446	0.119	1.562	3.74	**0.00018**
+*SCAR*	1.533	0.134	4.663	11.48	**<< 0.0001**
RAIN_GERMrescal	0.665	0.218	1.988	3.15	**0.00064**
+*SCAR : RAIN_GERMresc*	0.017	0.138	1.002	0.02	0.988
$SPPS_{A.tortilis}$ *: RAIN_GERMresc*	-0.140	0.123	0.789	-1.93	0.054
+*CAGE : RAIN_GERMresc*	-0.089	0.124	0.921	-0.66	0.508

‡Odds ratio of germination success and early seedling survival for each factor calculated as e^{β}.

Table 2. Effects of species identity (*A. tortilis* vs. *A. robusta*=control) caging (+*CAGE* vs. control -*CAGE*), water addition (+*WATER* vs. control -*WATER*), grass removal (-*GRASS* vs. control +*GRASS*) and scaled rainfall (*RAIN_SEEDL*) on the relative risk of juvenile seedling mortality (2-18 months post transplanting), inferred using a Cox-proportional hazards mixed effects model, and basal area growth rate ($mm^2\ yr^{-1}$), inferred from a general linearized mixed-effects model, of live transplanted juvenile seedlings across the Serengeti ecosystem. Note that in the Cox-proportional hazards model, coefficients increase positively with risk so that positive coefficients imply higher juvenile seedling mortality and negative coefficients imply lower juvenile seedling mortality.

	Juvenile seedling mortality risk					Juvenile seedling basal growth rate			
Factor	β	SE	exp(β)[†]	Z	P-value	β	SE	*T*	P-value
$SPPS_{A.tortilis}$	-0.946	0.180	0.388	-5.26	**< 0.0001**	10.70	2.68	3.99	**0.00**
+*CAGE*	-0.702	0.270	0.495	-2.61	**0.0091**	-0.74	2.61	-0.28	0.76
+*WATER*	0.125	0.169	1.133	0.74	0.46	4.40	5.26	0.84	0.39
+*GRASS*	1.190	0.233	3.288	5.12	**< 0.0001**	-1.48	4.79	-0.31	0.74
RAIN_SEEDL	0.600	0.203	1.822	2.96	**0.0031**	-0.41	1.83	-0.22	0.81
+*CAGE* : +*GRASS*	0.478	0.319	1.610	1.50	0.13	-4.88	5.17	-0.94	0.34
+*CAGE* : +*WATER*	-0.257	0.240	0.773	-1.07	0.29	-2.47	6.37	-0.39	0.68
$SPPS_{A.tortilis}$: +*GRASS*	0.732	0.191	2.079	3.83	**0.00013**	-9.52	4.01	-2.37	**0.02**

[†]Relative risk of treatment vs. control case (*e.g.*, +*CAGE* vs. -*CAGE*)

Figure legends

Fig. 1. (A) Tree life stages and experimental treatments. (B) Experimental design of subplots within plots. In the seed transplant study (Experiment 1), 16 seeds of either *A. tortilis* (T) and *A. robusta* (R) were planted in caged or uncaged, and either scarified (dotted) or un-scarified (white), subplots. In the seedling transplant experiment (Experiment 2), two seedlings of each species were planted in one of six different treatments per plot.

Fig. 2. Germination success and early seedling survival (0-2 months) increased with scaled rainfall and was higher inside herbivory cages (Experiment 1). Un-scarified seeds (A) had far lower germination success and early seedling survival than scarified seeds (B). Each point represents a subplot consisting of the mean success/survival of 16 seeds.

Fig. 3. Survivorship curves of transplanted juvenile seedlings over time for *Acacia robusta* (A) and *A. tortilis* (B) combined across all plots and cohorts (Experiment 2). Six experimental treatments include grass control/removal (±*GRASS*), caging/control (±*CAGE*) and water addition/control (±*WATER*). Note the water addition treatment was not crossed with grass removal.

Fig. 4. Survivorship of transplanted juvenile seedlings over time in the grass-addition experiment (Experiment 3). Results combined for *Acacia tortilis* and *A. robusta*.

Fig. 5. Predicted tree establishment (seed germination plus seedling survival from 0-18 months) and 95% confidence intervals (dotted lines) across rainfall for all combinations of significant experimental treatments: scarified (red) versus un-scarified seeds (blue), caged versus uncaged, *Acacia robusta* versus *A. tortilis* and seedlings grown in subplots with or without grass removal.

Fig. 1.

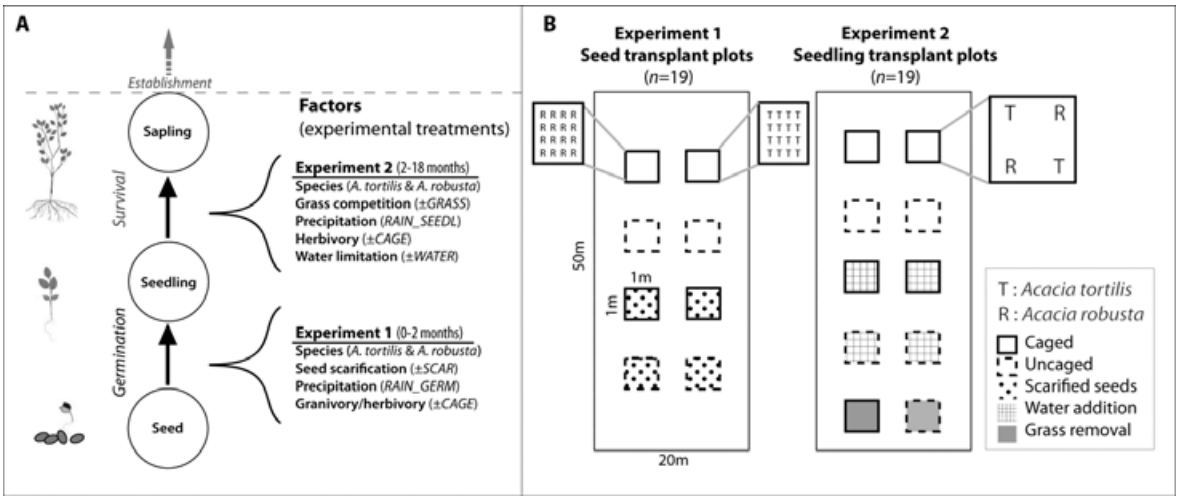

Fig. 2.

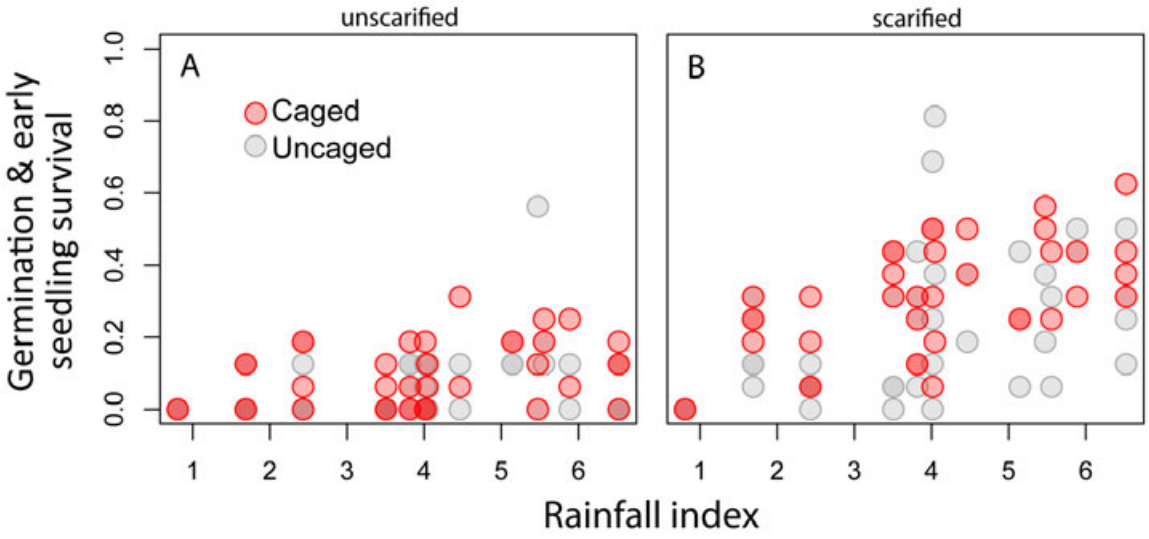

Fig. 3

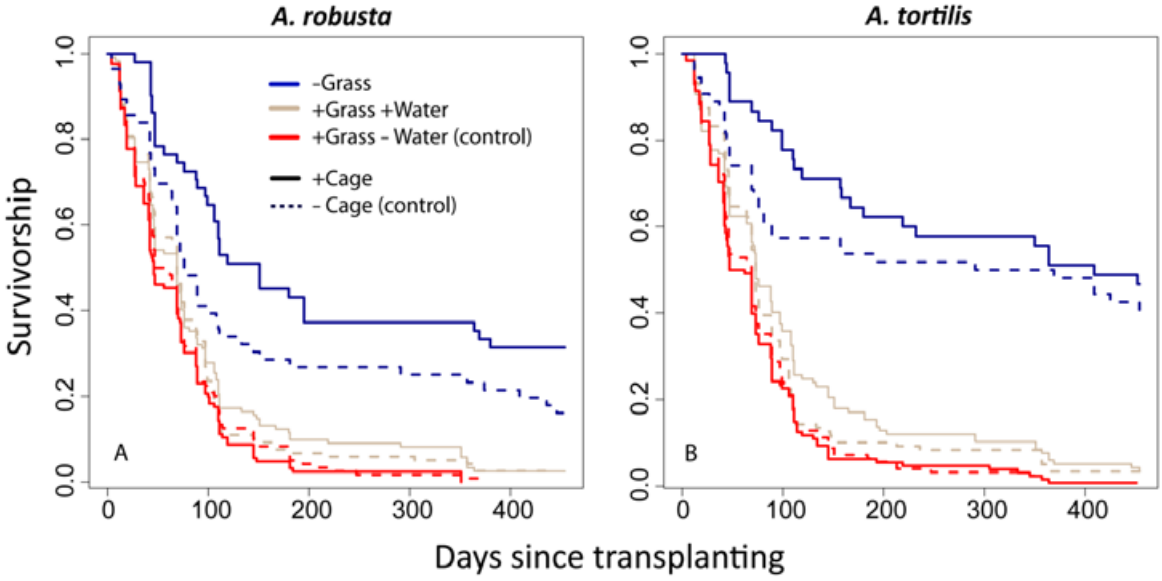

Fig. 4

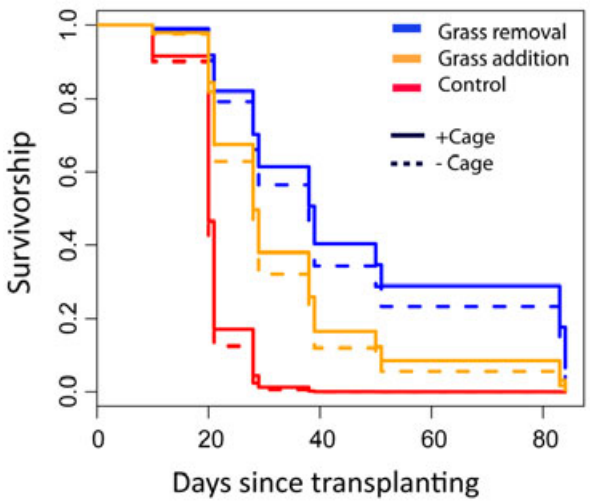

Fig. 5